

CLAIMS

I/WE CLAIM:

1. A modified α -glucosidase enzyme, the modified form differing from the wild-type barley α -glucosidase by proline being substituted at residue 340, the modified enzyme retaining activity at a higher temperature than the wild-type enzyme.
2. A DNA sequence which encodes the expression of the enzyme of claim 1.
3. A transgenic host which expresses the DNA sequence of claim 2 to produce the modified barley α -glucosidase.
4. A constructed DNA sequence including a protein coding region encoding a modified barley α -glucosidase enzyme, the modified barley α -glucosidase differing from the wild-type barley α -glucosidase by the presence of a proline residue at residue 340.
5. A transgenic host which expresses the constructed DNA sequence of claim 4.
6. A modified α -glucosidase enzyme, the modified form differing from the wild-type barley α -glucosidase by at least one amino acid substitution from the native barley α -glucosidase enzyme sequence, the modified enzyme retaining enzymatic activity at a higher temperature than the wild-type enzyme.

7. A modified α -glucosidase enzyme as claimed in claim 6 wherein the modification is selected from the group consisting of removing an aspartate at residue 83, removing an aspartate from residue 92, adding a proline to residue 100, adding a proline and removing an aspartate at residue 101, removing an aspartate from residue 105, adding a proline to residue 122, adding a proline to residue 184, removing N-glycosylation site from residue 298, adding a proline to residue 336, removing an aspartate from residue 369, adding N-glycosylation site and removing an aspartate from residue 372, removing N-glycosylation site from residue 391, adding a proline to residue 394, adding a proline and removing an aspartate at residue 403, adding N-glycosylation site to residue 463, removing an aspartate from residue 508, removing a deamidation site from residue 568, adding N-glycosylation site and removing an aspartate from residue 694, adding a proline at residue 713, adding a proline at residue 742, and removing an aspartate from residue 764.

8. A DNA sequence which encodes the modified α -glucosidase enzyme as claimed in claim 7.

9. A method of making a mutant form of the enzyme barley α -glucosidase comprising the steps of:

(a) constructing a mutant gene sequence encoding a mutant form of the α -glucosidase enzyme;

(b) cloning the mutant gene sequence into an expression vector;

(c) expressing the protein encoded by the expression vector to produce the protein encoded by the mutant gene sequence;

(d) recovering the protein produced; and

(e) testing the protein for both α -glucosidase activity and for thermostability; wherein the mutant gene sequence encoding a mutant protein has at least one mutation selected from the group consisting of removing an aspartate at residue 83, removing an aspartate from residue 92, adding a proline to residue 100, adding a proline and removing an aspartate at residue 101, removing an aspartate from residue 105, adding a proline to residue 122, adding a proline to residue 184, removing N-glycosylation site from residue 298, adding a proline to residue 336, removing an aspartate from residue 369, adding N-glycosylation site and removing an aspartate from residue 372, removing N-glycosylation site from residue 391, adding a proline to residue 394, adding a proline and removing an aspartate at residue 403, adding N-glycosylation site to residue 463, removing an aspartate from residue 508, removing a deamidation site from residue 568, adding N-glycosylation site and removing an aspartate from residue 694, adding a proline at residue 713, adding a proline at residue 742, and removing an aspartate from residue 764.